

Appl. No. : 09/919,758
Filed : July 31, 2001

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method for amplifying a transcriptionally-active polynucleotide, comprising:

PCR-amplifying a first target fragment of DNA with a first primer pair, wherein the first primer pair, upon such amplification, adds to first and second ends of the first fragment predetermined first and second regions of complementarity, to form a second DNA fragment having said first region of complementarity at a first end and a second region of complementarity at a second end of said second DNA fragment;

providing a promoter-containing sequence and a terminator-containing sequence, said promoter-containing sequence further including a region complementary to said first region of complementarity, and said terminator-containing sequence further including a region complementary to said second region of complementarity, wherein both said promoter-containing sequence and said terminator-containing sequence include an internal nucleotide capable of forming an A-T base pair immediately adjacent to said region of complementarity;

joining said promoter-containing sequence to said first end of said second DNA fragment and said terminator-containing sequence to said second end of said second DNA fragment to form said third DNA fragment; and

PCR-amplifying said third DNA fragment thereby generating a transcriptionally-active polynucleotide.

2. (Original) The method of claim 1, wherein said joining comprises joining in the presence of polymerase said promoter-containing sequence to said first end of said second DNA fragment and said terminator-containing sequence to said second end of said second DNA fragment to form said third DNA fragment.

3. (Previously Amended) The method of claim 1, wherein said promoter-containing sequence and said terminator-containing sequence further comprise a PNA binding domain.

4. (Cancelled)

5. (Previously Added) The method of claim 2, wherein said polymerase is a non blunt end polymerase.

Appl. No. : 09/919,758
Filed : July 31, 2001

6. (Previously Added) The method of claim 3, wherein the non blunt end polymerase is Taq polymerase.

7. (Currently Amended) The method of claim 1, wherein said PCR-amplifying comprises the addition of binding moiety to said third DNA fragment.

8. (Previously Added) The method of claim 7, wherein said binding moiety comprises a PNA molecule.

9. (Previously Added) The method of claim 7, wherein said binding moiety comprises at least one phosphorothioate.

10. (Previously Added) A method of adding a nucleic acid sequence that confers function to a polynucleotide target sequence, comprising:

contacting a first nucleic acid fragment with a polynucleotide target sequence, wherein the first nucleic acid fragment comprises a first region of complementarity to a portion of the polynucleotide target sequence and an extension region;

PCR amplifying the first nucleic acid fragment and the polynucleotide target, to form a second nucleic acid fragment that comprises the polynucleotide target sequence and the extension region;

contacting the second nucleic acid fragment with a third nucleic acid fragment that comprises a region complementary to the extension region and also a nucleic acid sequence that confers function; and

PCR amplifying the second nucleic acid fragment with the third nucleic acid fragment to form a fourth nucleic acid fragment that comprises the nucleic acid sequence that confers function joined to the polynucleotide target sequence.

11. (Currently Amended) The method of claim 10, wherein said region complementary to the extension region is not ~~specifically~~ complementary to a region on said polynucleotide target sequence.

12. (Previously Added) The method of claim 10, wherein the nucleic acid region that confers function comprises a promoter or a terminator.

13. (Cancelled)

14. (Currently Amended) The method of claim 131, wherein the PCR amplifying step is accomplished using a polymerase, and wherein the polymerase is a non blunt end polymerase.

Appl. No. : 09/919,758
Filed : July 31, 2001

15. (Previously Added) The method of claim 14, wherein the non blunt end polymerase is Taq polymerase.

16. (Previously Added) The method of claim 10, wherein said nucleic acid sequence that confers function and said second nucleic acid fragment include an internal nucleotide capable of forming an A-T base pair immediately adjacent to said extension region.

17. (Previously Added) The method of claim 10, wherein said step of PCR amplifying of the second nucleic acid fragment with the third nucleic acid fragment further comprises a primer, wherein said primer comprises a nuclease resistant binding moiety that upon amplification confers nuclease resistance to said fourth nucleic acid fragment.

18. (Previously Added) The method of claim 17, wherein said nuclease resistant binding moiety comprises a PNA molecule.

19. (Previously Added) The method of claim 17, wherein said nuclease resistant binding moiety comprises at least one phosphorothioate.

20. (Previously Added) The method of claim 10, wherein third nucleic acid fragment further comprises a PNA binding domain.

21. (Currently Amended) A method of adding a nucleic acid sequence that confers function to a polynucleotide target sequence, comprising:

contacting first and second nucleic acid fragments with a polynucleotide target sequence, wherein the first nucleic acid fragment comprises a first region of complementarity to a portion of the polynucleotide target sequence and a first extension region, and the second nucleic acid fragment comprises a second region of complementarity to a second portion of the polynucleotide target sequence and a second extension region;

PCR amplifying the first and second nucleic acid fragments and the polynucleotide target sequence, to form an intermediate nucleic acid fragment that comprises the polynucleotide target sequence flanked by the first and second extension regions;

contacting the intermediate nucleic acid fragment with third and fourth nucleic acid fragments that respectively comprise a region complementary to the first and second extension regions, wherein one or both of the third and forth fragments further comprise at least one nucleic acid region that confers function; and

Appl. No. : 09/919,758
Filed : July 31, 2001

PCR amplifying the intermediate nucleic acid fragment with the third and fourth nucleic acid fragments to form a product nucleic acid fragment that comprises one or more functional nucleic acid regions joined to the polynucleotide target sequence.

22. (Previously Added) The method of claim 21, wherein the product nucleic acid fragment comprise a promoter sequence or a terminator sequence or both.

23. (Currently Amended) The method of claim 21, wherein said functional product nucleic acid fragment further comprises a PNA binding domain.

24. (Currently Amended) The method of claim 21, wherein said PCR amplifying of the intermediate nucleic acid fragment further comprises a set of primers, wherein at least one primer comprises comprising one or more a nuclease resistant, binding moieties moiety that upon amplification confers nuclease resistance to said product nucleic acid fragment.

25. (Previously Added) The method of claim 24, wherein said one or more nuclease resistant, binding moieties comprise a PNA molecule or one or more phosphorothioate molecules or both.

26. (Cancelled)

27. (Currently Amended) The method of claim 2621, wherein said PCR amplifying steps are accomplished using a polymerase, and wherein said polymerase is a non blunt end polymerase.

28. (Previously Added) The method of claim 27, wherein said non blunt end polymerase is Taq polymerase.

29. (Previously Added) The method of claim 21, wherein said nucleic acid region that confers function comprises a promoter or a terminator.

30. (Currently Amended) A system for adding a nucleic acid fragment that confers function to a polynucleotide target sequence, comprising:

an extension primer pair, each primer of which comprises a region of complementarity to a strand of the polynucleotide target sequence and a predetermined extension region; and

a pair of 5' biological function conferring nucleic acid fragmentsfragment and a 3' biological function conferring nucleic acid fragment, each fragment of which comprises a region of complementarity to one of the extension regions, and a biological function conferring polynucleotide sequence that confers biological function, wherein the

Appl. No. : 09/919,758
Filed : July 31, 2001

extension primer pairs are adapted to add the extension regions to a target sequence upon a first PCR procedure, and the function conferring nucleic acid pairs are adapted to add the functional polynucleotide sequences to the polynucleotide target sequence upon a second PCR procedure.

31. (Previously Added) The system of claim 30, wherein the system further comprises a polymerase.

32. (Previously Added) The system of claim 31, wherein the polymerase is a non blunt end polymerase

33. (Previously Added) The system of claim 32, wherein the non blunt end polymerase is Taq polymerase.

34. (Currently Amended) The system of claim 30, wherein the 5' biological function conferring nucleic acid fragments comprises a promoter-and/or-a terminator.

35. (Previously Added) The system of claim 34, wherein the biological function conferring nucleic acid fragments further comprise at least one PNA binding domain.

36. (Previously Added) The system of claim 30, further comprising an additional primer pair comprising at least one nuclease-resistant, binding moiety.

37. (Previously Added) The system of claim 36, wherein the nuclease-resistant, binding moiety is selected from the group consisting of a PNA molecule and a phosphorothioate.

38. (Currently Amended) A method for creating transcriptionally-active nucleic acid sequences from a plurality of different target polypeptide-encoding DNA sequences, comprising:

creating extension primer pairs for each of a plurality of different target polypeptide-encoding sequences, each extension primer pair comprising first and second extension primers, respectively comprising first and second extension regions and a region of complementarity to a particular target sequence, such that the first and second extension regions for each extension primer pair are the same as the first and second extension regions for the other of said extension primer pairs, but the regions of complementarity are customized for each target sequence;

PCR-amplifying each of said target sequences with said extension primer pairs to provide intermediate sequences comprising said plurality of target sequences, each said target sequence flanked by the same first and second extension regions; and

providing transcriptionally-functional fragment pairs, wherein each transcriptionally-functional fragment pair comprises a first fragment having a region of complementarity to the first extension region and a second primer having a region of complementarity to the second extension region, and at least one of said fragments in said transcriptionally-functional fragment pair comprising a transcriptionally-functional region; and

PCR-amplifying each of said intermediate sequences with said transcriptionally-functional fragment pairs to provide a plurality of transcriptionally-functional polynucleotides, each comprising one of the target sequences linked to at least one transcriptionally-functional region.

39. (Previously Added) The method of claim 38, wherein said transcriptionally-functional region is a promoter or a terminator sequence.

40. (Previously Added) The method of claim 38, wherein said transcriptionally-functional fragment pair adds both a promoter and a terminator to the target sequence.

41. (Previously Added) The method of claim 38, wherein the PCR amplifications are performed separately for each of said target sequences.

42. (Cancelled)

43. (Currently Amended) The method of claim 4238, wherein said PCR-amplifying steps are accomplished using a polymerase, and wherein said polymerase is a non blunt end polymerase.

44. (Currently Amended) A method of generating a nuclease resistant ~~and functional~~ nucleic acid molecule, comprising:

contacting first and second nucleic acid fragments with a polynucleotide target sequence, wherein the first nucleic acid fragment comprises a first region of complementarity to a portion of the polynucleotide target sequence and a first extension region, and the second nucleic acid fragment comprises a second region of complementarity to a second portion of the polynucleotide target sequence and a second extension region;

PCR amplifying the first and second nucleic acid fragments and the polynucleotide target sequence, to form an intermediate nucleic acid fragment that

Appl. No. : 09/919,758
Filed : July 31, 2001

comprises the polynucleotide target sequence flanked by the first and second extension regions;

contacting the intermediate nucleic acid fragment with a third and a fourth nucleic acid fragments that respectively comprise a region complementary to the first and second extension regions and with a first primer and a second primer at least one of which comprises a nuclease-resistant, binding moiety, wherein one or both of the third and forth nucleic acid fragments further comprise a nucleic acid region that confers function; and

PCR amplifying the intermediate nucleic acid fragment with the third and fourth nucleic acid fragments and the first and second primers to form a nuclease resistant nucleic acid molecule that comprises one or more functional nucleic acid regions joined to the polynucleotide target sequence, and at least one nuclease-resistant, binding moiety on a 5' end.

45. (Previously Added) The method of claim 44, wherein, said nuclease-resistant, binding moiety is a PNA molecule and/or one or more phosphorothioate molecules.

46. (New) The system of claim 30, wherein the 3' biological function conferring nucleic acid fragments comprises a terminator.